North Clin Istanb 2026;13(3):327–335
doi: 10.14744/nci.2026.96337

Original Article Neurosurgery

Experimental sciatic nerve neuropathy caused by diclofenac sodium in rats

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ABSTRACT

OBJECTIVE: Our aim in this study is to investigate the total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) values of diclofenac sodium, which is a non-steroidal anti-inflammatory analgesic, after sciatic nerve injury, to examine the histopathological changes in the sciatic nerve, and to examine the results and enable important changes in treatment. It is one of the rare studies conducted histopathologically on post-injection sciatic nerve damage of diclofenac sodium, and the parameters of our biochemical approach have not been evaluated before in the literature.

METHODS: study was divided into 5 main groups as control, sham, trauma, isotonic fluid, and diclofenac sodium. Sham, trauma, isotonic fluid, and diclofenac sodium groups were divided into four subgroups as early stage (day 1, day 3) and late stage (day 7 and day 14). Subjects in each group were evaluated daily by neurological examination according to the Tarlov scale. In all groups except the control group, the sciatic nerve was exposed through appropriate surgical procedures. TAS, TOS, and OSI were run on blood samples biochemically. Nerve tissue samples were evaluated histopathologically and immunohistochemically.

RESULTS: There was a significant difference in pairwise comparison of the groups in terms of CASPASE-3 values. A statistically significant difference was found between the groups in terms of myelin thickness and axon diameter. A statistically significant difference was found between the groups in terms of nerve diameter values.

CONCLUSION: Our study demonstrates that intramuscular injections of diclofenac sodium have potential harmful effects. We recommend that alternative methods of using diclofenac sodium intramuscularly be preferred whenever possible due to the potential complications associated with this drug, that healthcare professionals receive necessary training on intramuscular administration, that the procedures be performed more carefully, and that patients be informed about these complications.

Keywords: Diclofenac sodium; injection; neuropathy; sciatic.

Cite this article as: *Aykut AM, Kaya ME, Oral S, Serarslan Y, Aras M, Yilmaz A, et al. Experimental sciatic nerve neuropathy caused by diclofenac sodium in rats. North Clin Istanb 2026;13(3):327–335.*

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Received: January 04, 2026 *Revised:* February 06, 2026 *Accepted:* June 25, 2026 *Online:* July 03, 2026

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Istanbul Provincial Directorate of Health - Available online at www.northclinist.com

Post-injection sciatic nerve damage is a serious health problem with socioeconomic and medicolegal dimensions in both developed and developing societies [1–3]. The general incidence is estimated to be around 2%. Affected patients may experience temporary and permanent neuropathic pain, sensory and motor deficits, and associated gait disturbances [4–6].

Injury after injection may vary depending on the neurotoxicity of the drug, the dose and rate of administration, and needle-related characteristics such as diameter and length [7]. Possible mechanisms of injury include chemical damage, neuronal ischemia, direct needle injury, compression injury, and constriction due to scarring. Free radicals and cytokines released as a result of some of the ischemic and inflammatory changes that occur after injury cause calcium ions to enter the cell. This leads to the activation of proteolysis sites [8]. Repairment after injury is a complex process involving changes in Schwann cells, activation of macrophages, and rearrangement of vascular structures [9, 10].

The aim of this study is to demonstrate histopathologically and biochemically the damage caused by diclofenac sodium, which induces nerve damage through oxidative stress and apoptosis mechanisms [11, 12]. To this end, our study used biochemical, histopathological, and functional approaches. In the biochemical approach, we evaluated total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) parameters. The parameters in our biochemical approach have not been evaluated in previous studies of sciatic nerve injury after injection in the literature.

Highlight key points

- Peripheral nerve injury is a common complication after intramuscular injection and can be prevented with training.
- Post-injection damage may vary depending on the neurotoxicity of the given drug, dose and rate of administration, and needle-related characteristics such as diameter and length.
- This is the first study conducted in this context.
- Post-injection sciatic nerve damages importance as a health problem with socioeconomic and medicolegal dimensions.
- It was shown that TOS and OSI values increased and TAS values decreased after diclofenac sodium injection.

MATERIALS AND METHODS

Experimental Model

Ethics committee approval was obtained from the local ethics committee. During the study, the study was conducted in accordance with the Declaration of Helsinki.

The use of experimental animals was carried out in accordance with local ethical guidelines. The study was divided into 5 main groups: Control, sham, trauma, isotonic fluid, and diclofenac sodium. The sham, trauma, isotonic fluid, and diclofenac sodium groups were divided into four subgroups: Early stage (day 1 and day 3) and late stage (day 7 and day 14). In our study, a total of 119 Wister albino rats (250–300 mg, male) were used, including the control and 7 rats in each subgroup, taking into account the statistical significance of any wastage that might occur. In all groups except the control group, the sciatic nerve was exposed through appropriate surgical procedures. In the sham groups, only the sciatic nerve was observed. Trauma groups underwent dry needling injury. Isotonic groups received 0.5 mL of intraneural isotonic solution. Diclofenac groups received 0.2 mg/kg/0.5 mL of diclofenac sodium intraneurally. The skin was then closed with 3/0 Prolene. We had to take blood and tissue samples on 1, 3, 7, and 14 days. Xylazine (30 mg/kg) and ketamine hydrochloride (200 mg/kg) were administered intraperitoneally for anesthesia.

Biochemical Evaluation

Sample Collection

Blood samples were collected from the rats intracardially into a yellow-capped gel biochemistry tube (BD Vacutainer SST II Advance). After allowing 20 min for clotting to occur, the samples were centrifuged for 10 min at 1500× g, and the supernatants were separated, aliquoted, and stored at -80°C until the day of the study for biochemical analysis.

Measurement of total Serum Antioxidant Status

The 2,2'-azinobis (3-ethylbenzothiazolin-6-sulfonate) (ABTS) reagent is radicalized with hydrogen peroxide in the presence of a buffer solution, while the pH of the environment is kept constant. The characteristic color (a color between dark green and dark blue) produced by the radical cation appears when the antioxidants in the sample are added to the medium, neutralizing the existing ABTS radicals. Therefore, the total amount of antioxidants in the serum is proportional to the color intensity of the solution. TAS values were expressed as mmol Trolox/L.

Measurement of TOS in Serum

When the oxidative species in the sample dissolve the compound Fe_2SO_4 in water, they release Fe_2^+. Oxidants present in the serum allow the Fe_2^+ to be oxidized to Fe_3^+. The ferric ion forms a colored complex with the X-orange reagent in an acidic environment. The intensity of the color, measured spectrophotometrically, is proportional to the total amount of oxidant molecules in the sample. TOS levels were expressed as μmol H_2O_2/L.

Calculation of the OSI

The OSI was calculated according to the following formula. OSI=TOS (μmol H_2O_2/L)/(TAS (mmol Trolox/L)×100) OSI was expressed as an arbitrary unit.

Histopathological Evaluation

Histomorphological Study

After the tissues were fixed in 10% formaldehyde solution for 48 h, 3 mm thick sections were cut perpendicular to the long axis of the nerve fiber and embedded vertically in paraffin. Sections of 4 microns were cut from each sample. After staining with toluidine blue, quantitative morphometric analyses were performed using a computerized image analysis program (Olympus BX51 microscope and DP2-BSW image analysis system). Immersion oil was used for measurements at high magnification (×1000). 15 nerve fibers were examined in each nerve section. Nerve diameter and axon diameter were measured. In addition, the ratio of axon diameter to nerve diameter (myelinated axon), the G-ratio, was calculated for each nerve fiber.

Immunohistochemical Study

Immunohistochemical staining was performed according to the protocol of the study entitled Effects of Dexamethasone on Bupivacaine-Induced Peripheral Nerve Injection Injury by Comez et al. [13].

Tonsil tissue was used as a positive control for anti-CASPASE 3 and S100, and schwannoma tissue was used as a positive control. All immunohistochemically stained specimens were examined blindly by the pathologist under a bright-field light microscope (Olympus BX53). To evaluate the percentage of CASPASE-3 positive cells, the percentage of positive nuclear staining with anti-CASPASE-3 was recorded in the nerve section of each rat. Brown positive cytoplasmic staining with S100 was observed in myelinated fibers as opposed to demyelinated fibers. The percentage of positive staining with S100 was determined using a four-point grading system as mild (+), moderate (++), severe (+++), and very severe (++++), according to the method of Bostan et al. [14]. Immunohistochemical staining was performed on the study groups and control cases at the same session.

Functional Analysis

Subjects in each group were assessed daily by neurological examination according to the modified Tarlov scale. On the Tarlov scale, Grade 0: Complete plegia, Grade 1: Marked hind limb movement but no weight bearing, Grade 2: Frequent or strong hind limb movement, marked hind limb movement not resulting in weight bearing or locomotion, Grade 3: Hind limbs support body weight but there is no normal walking, Grade 4: There is a slight loss of walking, Grade 5: It is judged as normal walking [15].

Statistical Analysis

The suitability of continuous variables included in the study was evaluated through graphical analysis and the application of the Shapiro–Wilk test. The results demonstrated that the continuous variables were not normally distributed, with the exception of TAS and G-ratio. Mean±standard deviation values were used in the presentation of descriptive statistics. Kruskal–Wallis non-parametric analysis of variance (ANOVA) was used to compare TOS, OSI, axon diameter, nerve diameter, myelin thickness, myelin number, Tarlov score, CASPASE-3, and S100 values between groups. Analysis results are presented with Bonferroni correction for pairwise comparisons. One-way ANOVA test was used to compare TAS and G-ratio values according to groups. When a difference was found as a result of the one-way ANOVA test, *post hoc* pairwise comparisons were performed to determine the different groups, and the analysis results were presented with Bonferroni correction. IBM Statistical Package for the Social Sciences Statistics 21.0 was used for statistical analyses and calculations. The level of statistical significance was accepted as $p<0.05$.

RESULTS

Biochemical Results

In the paired comparison of sham and control groups, a statistically significant increase in TOS was found only in the early sham group ($p=0.033$), while no significant difference was found in TAS and OSI. In the paired compar-

Table 1. Biochemical results of day 1 groups

Biochemical parameters	Control Mean±SD	Sham-1 Mean±SD	Isotonic-1 Mean±SD	Trauma-1 Mean±SD	Diclofenac-1 Mean±SD	Test statistic	
						χ^2; F	p
TAS	1.14±0.17	1.03±0.25	0.97±0.26	0.94±0.24	0.95±0.39	F=0.623	0.650
TOS	8.11±3.26	15.03±2.98	13.73±3.49	20.34±5.27	17.39±4.80	χ^2=17.652	**0.001***
OSI	0.73±0.34	1.52±0.43	1.48±0.53	2.45±1.39	2.04±0.88	χ^2=16.835	**0.002***

TAS: Total antioxidant status; TOS: Total oxidant status; OSI: Oxidative Stress Index; F: One-way analysis of variance; χ^2: Kruskal–Wallis test statistic; SD: Standard deviation; *: P<0.05 statistical significant value.

Table 2. Biochemical results of day 3 groups

Biochemical parameters	Control Mean±SD	Sham-2 Mean±SD	Isotonic-2 Mean±SD	Trauma-2 Mean±SD	Diclofenac-2 Mean±SD	Test statistic	
						χ^2; F	p
TAS	1.14±0.17	1.01±0.20	1.01±0.24	0.98±0.39	0.95±0.22	F=0.535	0.711
TOS	8.11±3.26	10.82±8.20	11.59±4.46	16.26±3.64	15.02±2.84	χ^2=12.813	**0.012***
OSI	0.73±0.34	1.17±0.96	1.16±0.45	1.89±0.85	1.63±0.32	χ^2=11.852	**0.018***

TAS: Total antioxidant status; TOS: Total oxidant status; OSI: Oxidative Stress Index; F: One-way analysis of variance; χ^2: Kruskal–Wallis test statistic; SD: Standard deviation; *: P<0.05 statistical significant value.

ison of trauma and control groups, statistically significant increases in TOS (p<0.001, p=0.015) and OSI (p=0.001, p=0.017) were found in the early trauma group compared to the control group. In the paired comparison between isotonic groups and the control group, no statistically significant difference was found in TAS, TOS, and OSI values. In the pairwise comparison of diclofenac groups with the control group, statistically significant increases in TOS (p=0.011, p=0.021) and OSI (p=0.005, p=0.017) were found in the early diclofenac groups compared to the control group. However, a significant decrease in TOS (p=0.012, p=0.023) and OSI (p=0.008, p=0.025) was observed in the late diclofenac group compared to the early diclofenac groups. In the pairwise comparison of the control and early groups (Table 1, 2), a significant increase in TOS (p=0.001, p=0.017) and OSI (p=0.002, p=0.009) values was observed in the trauma and diclofenac groups compared to the control group. In the pairwise comparison of the control and late-stage groups, a significant increase in TOS (p=0.042, p=0.006) and OSI (p=0.013, p=0.042) values was observed between the control and trauma groups. However, a greater decrease in TOS (p=0.006) was observed in the late-stage diclofenac group compared to the trauma group.

Histomorphological Results

In our study, axon diameter, nerve diameter, G-ratio, myelin thickness, and myelin count were evaluated as histological parameters. In the pairwise comparison of the control and sham groups, a statistically significant decrease in myelin thickness (p=0.015, p=0.048, and p=0.037) and myelin count (p=0.007, p=0.034, and p=0.005) was observed in the sham group compared to the control group in both the early and late periods. In the pairwise comparison of the trauma and control groups, a significant decrease in nerve diameter (p=0.004) and myelin thickness (p=0.001) was observed in the trauma group compared to the control group in the early period. A significant decrease in myelin count was observed as the days progressed (Fig. 1A). In the pairwise comparison between the isotonic groups and the control group, a significant decrease in axon diameter was observed in the early period (p=0.017) and a significant increase in the late period (p=0.021) compared to the control group. A significant decrease in myelin thickness (p=0.009) was observed in the early period compared to the control group, while myelin count decreased in both the early and late periods (p<0.001, p=0.014) (Fig. 1B). A significant

TABLE 3. Histomorphological results of day 1 groups

Measured parameter	Control Mean±SD	Sham-1 Mean±SD	Isotonic-1 Mean±SD	Trauma-1 Mean±SD	Diclofenac-1 Mean±SD	Test statistic	
						χ^2; F	p
Axon diameter	2688.17±678.16	2513.57±667.95	2160.00±467.27	1963.88±478.04	3013.60±828.77	χ^2=6.215	0.184
Nerve diameter	6469.51±916.96	5556.00±712.91	4860.63±541.15	5050.86±594.02	5338.08±417.52	χ^2=12.675	**0.013***
G-ratio	0.41±0.06	0.44±0.06	0.43±0.07	0.38±0.05	0.55±0.13	F=4.263	**0.008***
Myelin thickness	2005.00±319.72	1520.71±158.19	1349.86±174.43	1543.14±87.04	1162.80±324.86	χ^2=19.698	**0.001***
Myelin count	24.00±2.58	20.43±4.16	7.86±1.46	17.00±4.58	7.00±1.41	χ^2=26.572	**<0.001***
CASPASE-3	0.14±0.38	0.43±0.53	4.57±2.76	4.43±2.37	5.00±5.21	χ^2=23.888	**<0.001***
S100	4.00±0.00	3.57±0.53	3.71±0.49	3.14±0.90	2.83±0.98	χ^2=9.092	0.059

F: One-way analysis of variance; χ^2: Kruskal–Wallis test statistic; SD: Standard deviation; *: P<0.05 statistical significant value.

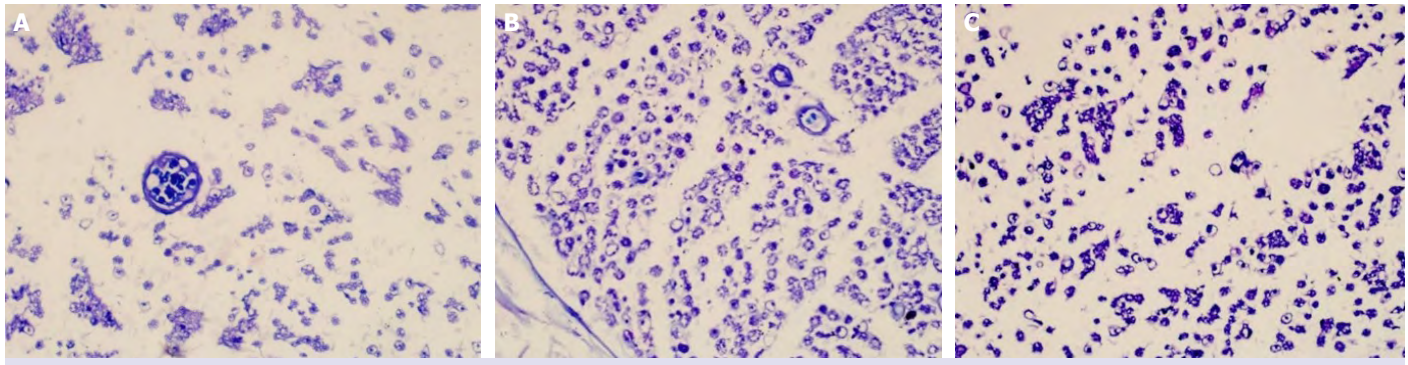

FIGURE 1. **(A)** In the trauma group, loss and damage to the myelin sheath and decrease in myelin number (Toluidine blue ×200). **(B)** Partial loss, degeneration, and thinning of the myelin sheath in the isotonic group (Toluidine blue ×200). **(C)** Decrease in the number of myelinated axons, thinning and damage in the sheath thickness in the diclofenac group (Toluidine blue ×200).

increase in the G-ratio was observed within the group as the days progressed. In the pairwise comparison between the diclofenac groups and the control group, a statistically significant decrease in myelin thickness (p=0.010, p=0.001) and myelin count (p=0.003, p=0.001) was observed in both the early and late periods compared to the control group. A significant increase in axon diameter (p=0.004) was observed in the late period (Fig. 1C). Furthermore, a statistically significant increase in the G-ratio was observed as the days progressed. In the comparison of the control and early-stage groups (Table 3, 4), myelin thickness was decreased in the trauma, isotonic, and diclofenac groups, while myelin count was significantly decreased in the isotonic and diclofenac groups. Furthermore, myelin thickness and count were significantly decreased in the isotonic and diclofenac groups compared to the sham group. The G-ratio was significantly increased in the trauma group, especially in the isotonic and diclofenac groups. Nerve diameter was significantly increased in the diclofenac group (p=0.029), and axon diameter was also found to be increased (p=0.001).

In the comparison of the control and late-stage groups (Table 5, 6), axon diameter was significantly increased in the diclofenac group (p=0.015). Myelin thickness was significantly decreased in the isotonic (p=0.044) and diclofenac (p<0.001) groups, while myelin count was significantly decreased in the trauma (p=0.008), isotonic (p=0.006), and diclofenac (p<0.001) groups. This decrease was more pronounced in the diclofenac group. The G-ratio was found to be increased in the trauma

Table 4. Histomorphological results of the day 3 groups

Measured parameter	Control Mean±SD	Sham-2 Mean±SD	Isotonic-2 Mean±SD	Trauma-2 Mean±SD	Diclofenac-2 Mean±SD	Test statistic	
						χ^2; F	p
Axon diameter	2688.17±678.16	2417.43±528.86	2969.46±920.65	1992.28±249.64	3779.80±543.15	χ^2=17.150	**0.002***
Nerve diameter	6469.51±916.96	5173.53±763.04	5091.54±1064.14	4495.60±562.16	5968.80±545.69	χ^2=16.555	**0.002***
G-ratio	0.41±0.06	0.46±0.06	0.56±0.08	0.45±0.07	0.62±0.05	F=12.687	**<0.001***
Myelin thickness	2005.00±319.72	1377.67±213.36	1060.57±157.02	1251.14±300.36	1094.00±212.04	χ^2=19.779	**0.001***
Myelin count	24.00±2.58	18.00±4.52	6.00±1.83	8.86±2.19	5.86±1.86	χ^2=26.948	**<0.001***
CASPASE-3	0.14±0.38	0.50±0.55	5.57±3.36	4.71±1.70	4.29±3.04	χ^2=23.628	**<0.001***
S100	4.00±0.00	3.33±0.82	3.57±0.53	2.86±1.07	3.43±0.79	χ^2=7.866	0.097

F: One-way analysis of variance; χ^2: Kruskal–Wallis test statistic; SD: Standard deviation; *: P<0.05 statistical significant value.

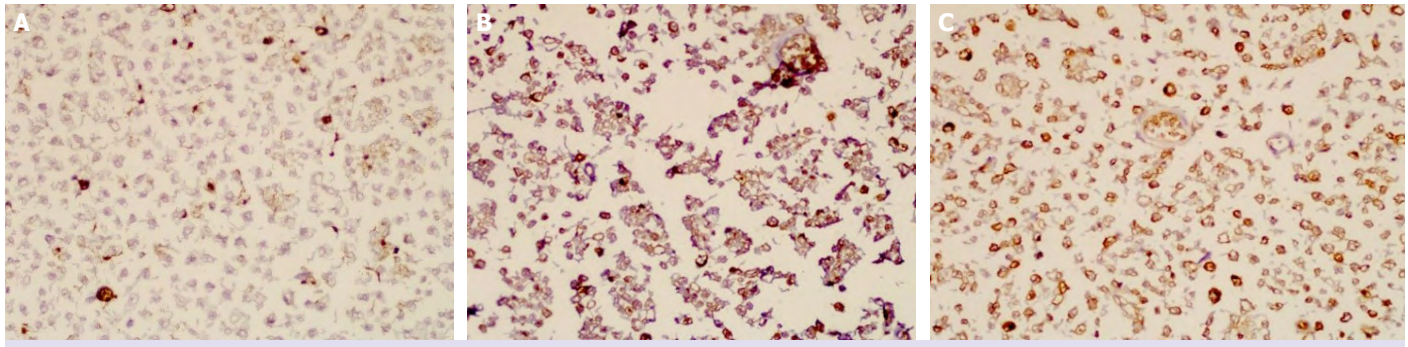

Figure 2. **(A)** The highest Caspase 3 activation in the diclofenac-treated group (Caspase 3 ×200). **(B)** S100 showed similar staining pattern among diclofenac-treated groups (S100 ×200). **(C)** S100 expression was similar between the isotonic group and the control group (S100 ×200).

group, and more pronounced in the isotonic (p=0.006) and diclofenac (p<0.001) groups.

Immunohistochemical Results

In the pairwise comparison of the sham and control groups, no statistically significant difference was observed in CASPASE-3 values. In the pairwise comparison between the trauma groups and the control group, a significant increase in CASPASE-3 values was observed in the trauma groups in both the early and late periods (p=0.026, p=0.003, p=0.005). In the pairwise comparison of the isotonic groups, a significant increase in CASPASE-3 was observed in the early period compared to the control group (p=0.009, p=0.002). In the pairwise comparison between the diclofenac groups and the control group, a significant increase in CASPASE-3 was observed in both the early and late periods (p=0.033, p=0.001) (Fig. 2A).

In the comparison of the control and early-stage groups (Table 3, 4), a significant increase in CASPASE-3 was observed in the trauma (p=0.005), isotonic (p=0.005), and diclofenac (p=0.026) groups compared to the control group. This increase was more pronounced in the diclofenac group, but also in the isotonic group. In the comparison of the control and late-stage groups (Table 5, 6), a significant increase in CASPASE-3 was observed in the trauma (p=0.001), isotonic (p=0.028), and diclofenac (p=0.001) groups compared to the sham and control groups. When S-100 values were evaluated, a significant decrease was observed only in the late-stage

Table 5. Histomorphological results of the day 7 groups

Measured parameter	Control Mean±SD	Sham-3 Mean±SD	Isotonic-3 Mean±SD	Trauma-3 Mean±SD	Diclofenac-3 Mean±SD	Test statistic	
						χ^2; F	p
Axon diameter	2688.17±678.16	2776.74±728.46	3590.11±853.71	2533.20±1034.21	3412.97±489.07	χ^2=7.148	0.128
Nerve diameter	6469.51±916.96	5661.97±511.68	5968.17±1147.51	5615.67±1338.76	5909.73±524.97	χ^2=3.357	0.500
G-ratio	0.41±0.06	0.48±0.11	0.58±0.04	0.43±0.09	0.57±0.05	F=7.383	**<0.001***
Myelin thickness	2005.00±319.72	1442.29±257.17	1188.71±185.76	1540.83±301.64	1248.00±143.39	χ^2=19.338	**0.001***
Myelin count	24.00±2.58	19.57±3.69	7.86±1.95	8.43±3.73	6.86±1.68	χ^2=25.610	**<0.001***
CASPASE-3	0.14±0.38	0.86±0.69	3.43±2.29	6.00±3.16	2.71±2.36	χ^2=19.662	**0.001***
S100	4.00±0.00	3.29±0.95	3.71±0.49	2.57±0.98	3.57±0.53	χ^2=12.126	**0.016***

F: One-way analysis of variance; χ^2: Kruskal–Wallis test statistic; SD: Standard deviation; *: P<0.05 statistical significant value.

Table 6. Histomorphological results of the day 14 groups

Measured parameter	Control Mean±SD	Sham-4 Mean±SD	Isotonic-4 Mean±SD	Trauma-4 Mean±SD	Diclofenac-4 Mean±SD	Test statistic	
						χ^2; F	p
Axon diameter	2688.17±678.16	3054.17±650.07	3341.68±569.29	2365.77±399.83	4368.34±744.07	χ^2=18.311	**0.001***
Nerve diameter	6469.51±916.96	5932.86±1110.68	6026.97±1120.51	5837.77±719.46	6430.00±738.69	χ^2=4.974	0.290
G-ratio	0.41±0.06	0.51±0.07	0.54±0.05	0.41±0.05	0.67±0.08	F=20.224	**<0.001***
Myelin thickness	2005.00±319.72	1438.86±317.26	1342.14±347.51	1735.67±286.19	1016.14±277.46	χ^2=20.558	**<0.001***
Myelin count	24.00±2.58	18.00±2.00	7.29±1.38	7.00±1.79	5.57±1.51	χ^2=27.059	**<0.001***
CASPASE-3	0.14±0.38	1.14±0.90	3.57±3.41	6.57±4.39	8.57±6.75	χ^2=23.148	**<0.001***
S100	4.00±0.00	3.43±0.79	3.71±0.49	3.14±0.90	3.29±0.76	χ^2=6.927	0.140

F: One-way analysis of variance; χ^2: Kruskal–Wallis test statistic; SD: Standard deviation; *: P<0.05 statistical significant value.

trauma group (p=0.026). However, there was no statistically significant difference between the early and late stages in the comparison of the sham, isotonic, and diclofenac groups (Fig. 2B, C).

Functional Results

The subjects were found to be neurologically sound before the procedure. Motor deficits developed in some subjects in the trauma, isotonic, and diclofenac groups. The severity of motor deficits was greater in the diclofenac groups. However, in our study, no statistically significant difference was found in the pairwise comparison of the groups in functional evaluation.

DISCUSSION

Nerve damage after intramuscular injections is common, and these injections are widely used in the treatment of diseases. Although the incidence of injection neuropathy has decreased in developed societies in the last decade, it

remains a serious health problem in both developed and developing countries. Ischemic, toxic, and mechanical causes play a role in the pathogenesis of post-injection neuropathy. While injection neuropathy is clinically variable, it generally manifests as pain, sensory deficits, and motor loss [16]. Diclofenac sodium has been reported as one of the most frequently used agents for intramuscular injections. Diclofenac sodium has been shown to be a neurotoxic agent in many studies [1, 14, 17].

Although many molecules associated with neuronal damage and repair have been identified, the mechanism is not fully understood. Oxidative stress is thought to be responsible for the damage that occurs after injury. Many antioxidants are found in cells and tissues to prevent oxidative damage by scavenging reactive oxygen species [18]. In this study, the biochemical approach evaluated TAS, TOS, and OSI. There are no studies on post-injection sciatic nerve damage. In our study, when the groups were compared with each other, it was found that TOS and OSI levels decreased gradually but were higher than the control group. In addition, it was determined that TAS levels between the groups were statistically insignificantly lower than the control group. It has been suggested that low TAS levels may be associated with fibrosis.

In our study, when the early groups were compared, a significant increase in TOS ($p=0.001$, $p=0.017$) and OSI ($p=0.002$, $p=0.009$) was observed in the trauma and diclofenac groups. When the late groups were compared, a significant increase in TOS and OSI levels was found only in the trauma group ($p=0.007$, $p=0.013$). It has been suggested that high serum TOS and OSI levels may be associated with fibrosis [19, 20].

Axon diameter, nerve diameter, G-ratio, myelin thickness, and myelin count, which we used as histological parameters in our study, are still important markers in evaluating regeneration after nerve injury [21]. Previous studies have associated degeneration with a low G-ratio value; it has been emphasized that a high G-ratio value indicates regeneration or demyelination in myelinated nerves [22].

When we compared the groups in our study, it was found that the myelin count and thickness decreased compared to the control group. The G-ratio was shown to gradually increase in the sham, isotonic, and diclofenac groups. In addition, it was observed that the axon diameter gradually increased in the isotonic and diclofenac groups. Nerve diameters were found to be lower compared to the control group. These results show that there was more damage in the diclofenac and isotonic groups as the days progressed. In the pairwise comparison of the early-stage groups in our study, the most significant decrease in myelin count and thickness was observed in the diclofenac and isotonic groups, indicating that degeneration was greater in these groups. In addition, the diclofenac group showed the greatest increase in G-ratio and axon diameter, suggesting that the damage was more severe than in the isotonic group. When comparing the late-stage groups, the G-ratio increased more in both the diclofenac and isotonic groups, and myelin thickness decreased more in the diclofenac group. Furthermore, myelin count decreased more in the diclofenac group. Our early and late-stage results demonstrate that degeneration and regeneration are more pronounced in the diclofenac group. Furthermore, it shows that the damage was more severe in the diclofenac group compared to the isotonic group.

Caspase-3 is known to play a significant role in dose-dependent apoptosis, and S-100 is a biomarker associated with axon diameter and myelination in Schwann cells and may reflect the regeneration phase [13, 23]. According to Bostan et al. [14], the S-100 percentage value is classified as mild (0–20, +), moderate (20–40, ++), severe (40–60, +++), and very severe (>60, ++++) positivity. When we compared the CASPASE-3 level groups in our study, the S-100 score was higher and lower compared to the control group. When we compared the early-stage groups, a significant increase in CASPASE-3 levels was found in the diclofenac, isotonic, and trauma groups. There was an insignificant decrease in S-100 levels. We hypothesized that high CASPASE-3 and low S-100 levels would be associated with a decrease in Schwann cell number. Furthermore, low S-100 levels are associated with drug toxicity. When comparing the late-stage groups, a significant increase in CASPASE-3 levels was observed in the trauma, isotonic, and diclofenac groups, respectively. This indicated that apoptosis was higher in the diclofenac group and more toxic than in the isotonic group. The diclofenac sodium used in our study was the lowest dose reported in the literature, and our histopathological findings were consistent with those of other researchers [13, 14]. No statistically significant difference was found in the functional evaluation of our study.

The Limitations of this Study

Electrophysiological methods were not included in the evaluation of sciatic nerve injury in this study.

Conclusion

Post-injection sciatic nerve damage can result in a clinical picture ranging from permanent and temporary pain to loss of strength. Therefore, it is considered a significant health problem with socioeconomic and medico-legal dimensions. This study demonstrates the potential harmful effects of intramuscular injections of diclofenac sodium, frequently prescribed by physicians, on peripheral nerves using histopathological and biochemical methods. Due to the potential complications associated with this drug, we recommend that alternative methods be preferred for intramuscular use of diclofenac sodium whenever possible, that healthcare professionals receive the necessary training on intramuscular administration, that procedures be performed more carefully, and that patients be informed about these complications.

Ethics Committee Approval: This study was approved by The Ethics Committee for Animal Experiments of Hatay Mustafa Kemal University Rectorate (2021/04-03 – 18/06/2021).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

Conflict of Interest: The authors declare that there is no conflict of interest.

Financial Disclosure: The authors declared that this study received no financial support.

Use of AI for Writing Assistance: The authors declared that artificial intelligence was not used in the study.

Authorship Contributions: Concept – YS; Design – MA; Supervision – AMA; Resource – TO, SD; Materials – AY; Data Collection and/or Processing – SO; Analysis and/or Interpretation – MEK; Literature Search – MEK; Writing – AMA; Critical Reviews – AM.

Peer-review: Externally peer-reviewed.

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